Efforts Towards the Synthesis of a Molecularly Imprinted Matrix as a Phosphonated Acetylcholine Mimic

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Abstract

The goal of my research has been to develop a molecularly imprinted polymer matrix that mimics the transition state of acetylcholinesterase binding to a phosphonate. The monomer is based around a modified styrene 'anchor' attached to a 'linker' segment, which is then attached to choline-bound phosphonate. Upon polymerization, the phosphonate will be removed and the exposed pockets of the matrix will be converted to a hydrated aldeyhyde to facilitate future bindings to substrates.

Acetylcholine, Acetylcholinesterase, and Organophosphonates

Acetylcholine (ACh) is a neurotransmitter responsible for muscular contraction and is located in the brain, spinal cord, and parts of the peripheral nervous system. Acetylcholine is synthesized in the body by choline acetyltransferase (ChAT) reacting with acetyl-coenzyme A (Acetyl-CoA). Acetylcholine is deactivated by the enzyme acetylcholinesterase (AChE), which is located in the post-synaptic membrane. The manipulation of acetylcholine and acetylcholinesterase is a key operating function of many toxins. Additionally, several medical conditions exist wherein acetylcholinesterase inhibitors become necessary for normal function.[1]

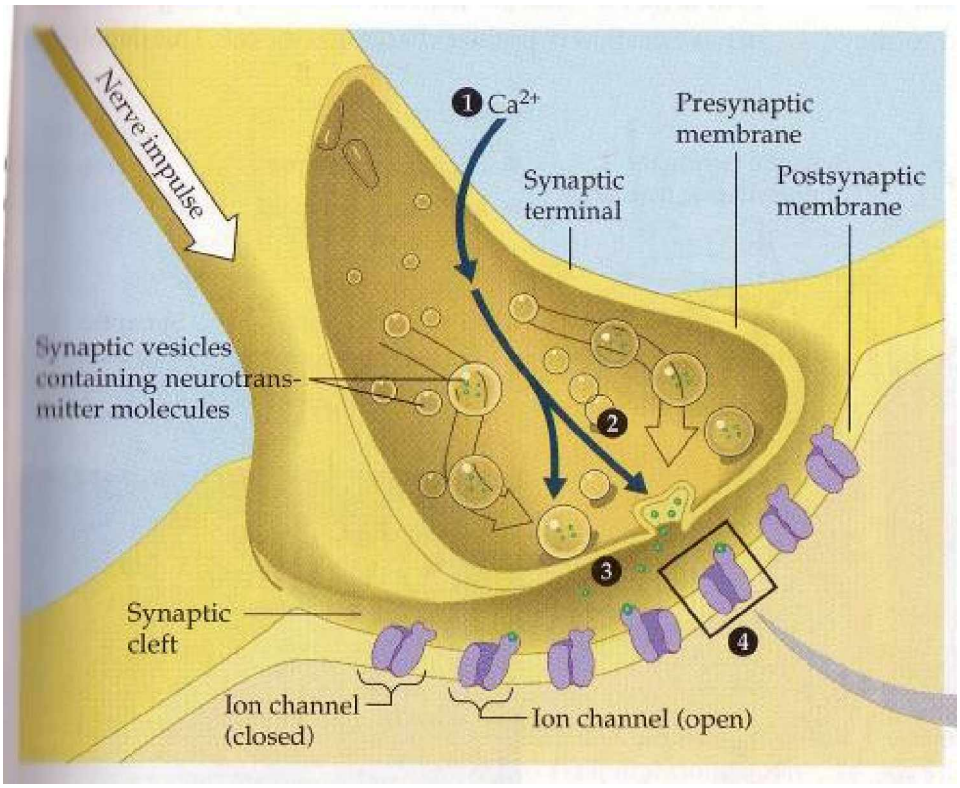


Figure 1: Nerve Synapse Diagram[2]

Acetylcholinesterase in normal biological systems acts by deactivating acetylcholine via rapid hydrolysis. In addition to the regulation of acetylcholine, acetylcholinesterase also affects cell proliferation, differentiation, as well as cellular stress response.[3]

As a class of chemicals, organophosphonates have been successfully applied as acetycholinesterase inhibitors. Practical applications include use as pesticides and chemical warfare agents, as well as implementation as therapeutic agents for neurological conditions such as Alzheimer's disease, Glaucoma, and Myasthenia Gravis.[4]

The inhibition of acetylcholinesterase results in unnaturally high levels of acetylcholine. This in turn causes an over-stimulation of the cholinergic receptors. Symptoms of organophosphonate exposure are muscarinic, nicotinic, central nervous system disruption, as well as adverse reproductive effects (Table 1). It is precisely this toxicity from over-stimulation that is exploited in the use of organophosphate agents as pesticides and chemical warfare agents (Sarin, VX).[4]

The commonly agreed upon function of inhibition for organophosphate pesticides (and by association, chemical warfare agents) is the phosphorylation of acetylcholinesterase at the serine 200 residue via fluoride replacement.[3,4] Figure 2 illustrates the key amino acid residues of acetylcholinesterase. Figure 3 demonstrates the phosphorylation of acetylcholinesterase at the serine 200 residue by cyclosarin, a potent organophosphate classified as a biochemical weapon.

Table 1: Organophosphate Exposure Effects[4,5]

Muscarinic Effects

- Nausea
- Vomiting
- Heart Block
- Pulmonary Edema
- Abdominal Cramps

Nicotinic Effects

- Hypertension
- Weakness
- Muscle Fasciculation

Central Nervous System Effects

- Confusion
- Headache
- Tremor
- Convulsions
- Coma
- Respiratory Depression

Visible Physical Symptoms

- Salivation
- Lacrimation
- Miosis
- Hypothermia
- Mild Tremors
- Mouthasmacking
- Lowered Motor Activity
- Decreased Tail-Pinch Response
- Altered Neuromuscular Function

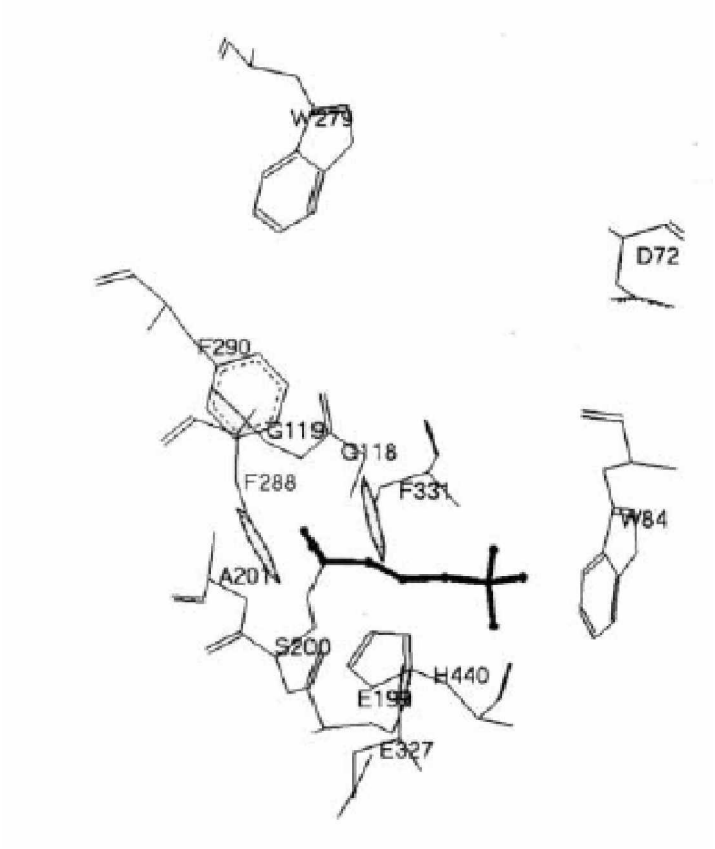


Figure 2: Key Acetylcholinesterase Residues and Bound Acetylcholine[3]

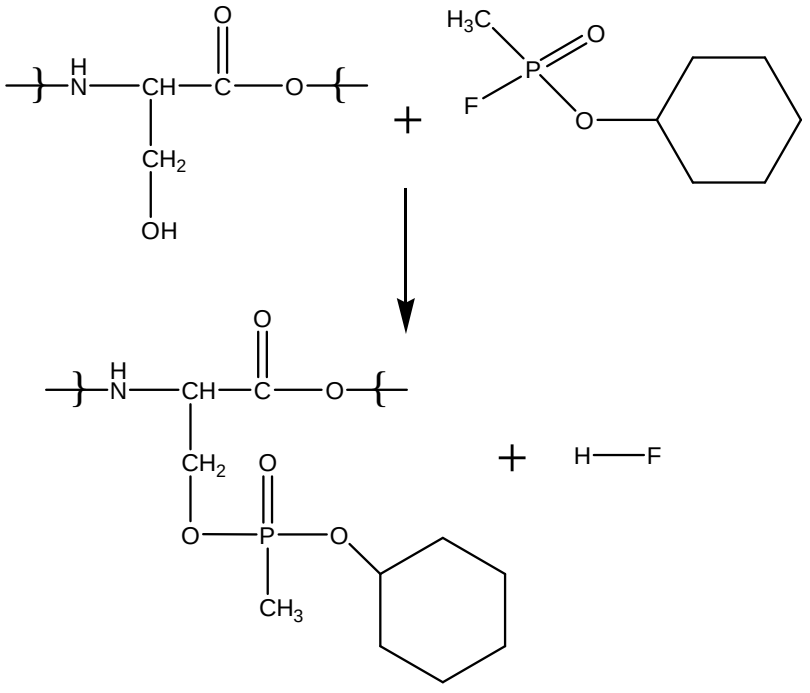


Figure 3: Cyclosarin Binding to Acetylcholinesterase Serine 200 Residue[5]

Dougherty and coworkers have studied the binding of acetylcholine to both natural and synthetic receptors.[6,7] They have determined that the primary binding action involves a pi-cation interaction between the receptor and the quaternary trimethyl ammonium of choline (See Figure 4).

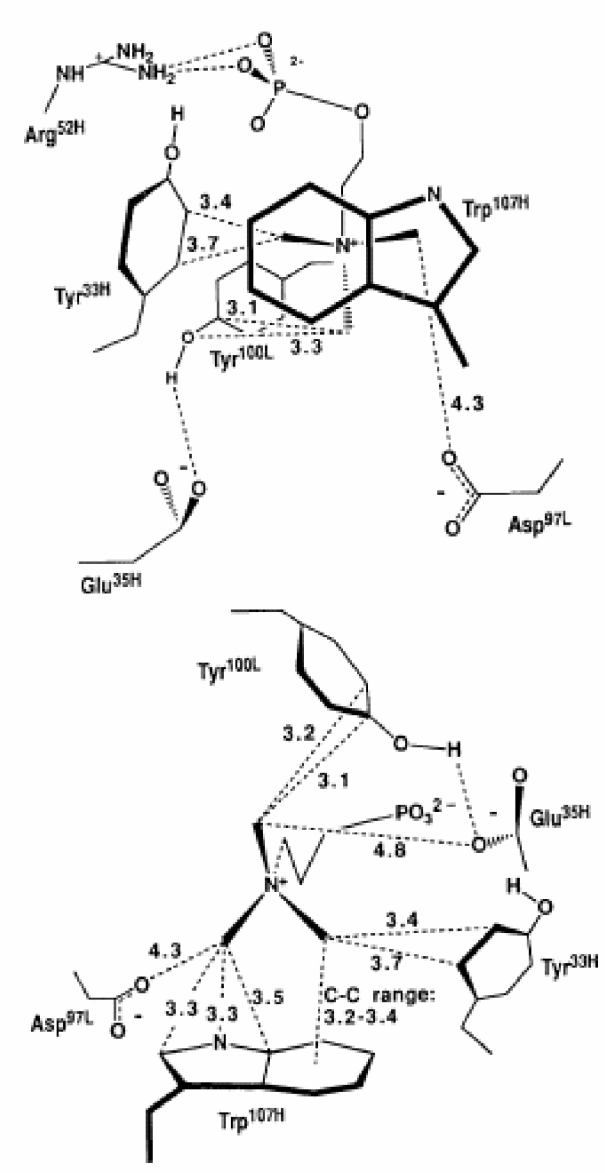


Figure 4: Acetylcholine binding to key Residues of Immunoglobin Fab McPC603[7]

Solid Phase Organic Synthesis, *J*anda*J*el, and Molecularly Imprinted Polymers

Polystyrene-based resins have proved to be a useful field for the development of solid phase organic synthesis (SPOS). *J*anda*J*el™ continues to be a platform of interest; it is believed that the presence of ether linkages results in the observed superior swelling characteristics upon exposure to a variety of solvents. This results in reaction kinetics superior to that observed in Merrifield resins.8-11

Molecularly imprinted polymers (MIP) are an area of increasing interest to synthetic chemists. Rather than simply serving as a media within which to conduct reactions, a polymer matrix forms around a template molecule. When the template is subsequently removed, 'pockets' remain within the matrix corresponding to the shape of interest dictated by the template. Applied as such, imprinted polymers have been successfully employed as coenzyme analogs.[12]

Research Interests and Project Planning

The primary goal of this research project is to develop an imprinted polymer that mimics the transition state of phosphorylated acetylcholinesterase. The target molecule will exhibit a polymerizable anchor, a 'linker' and a phosphonate-choline group (see Figure 5). Success will require the use of a polymer base that can provide the required pi-cation interaction between the quaternary trimethyl ammonia of choline. Furthermore, the end polymer matrix must exhibit suitable swelling properties.

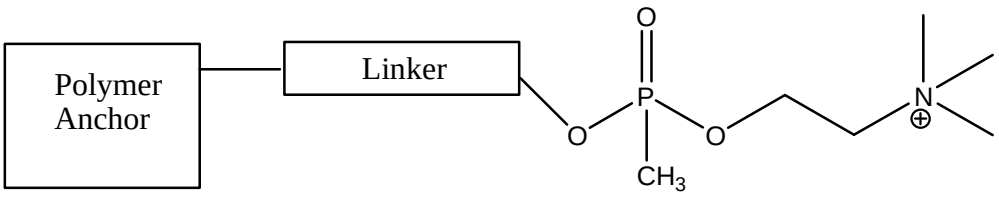


Figure 5: General Molecule Scheme

A styrene anchor is a logical choice, as it will be readily polymerized to form the imprinted matrix upon completion of target molecule. Multiple ether linkages within the 'linker' should provide enhanced swelling properties in the final matrix.

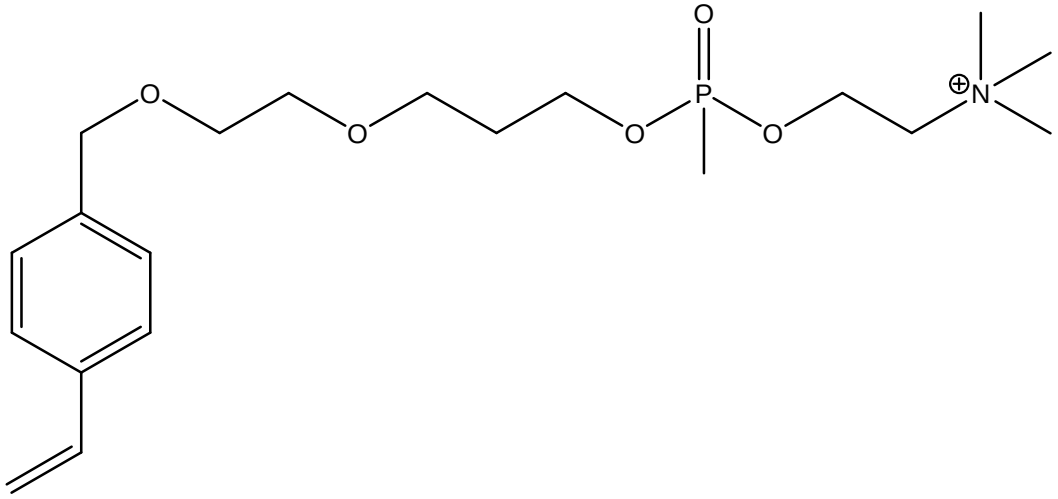


Figure 6: Target Molecule

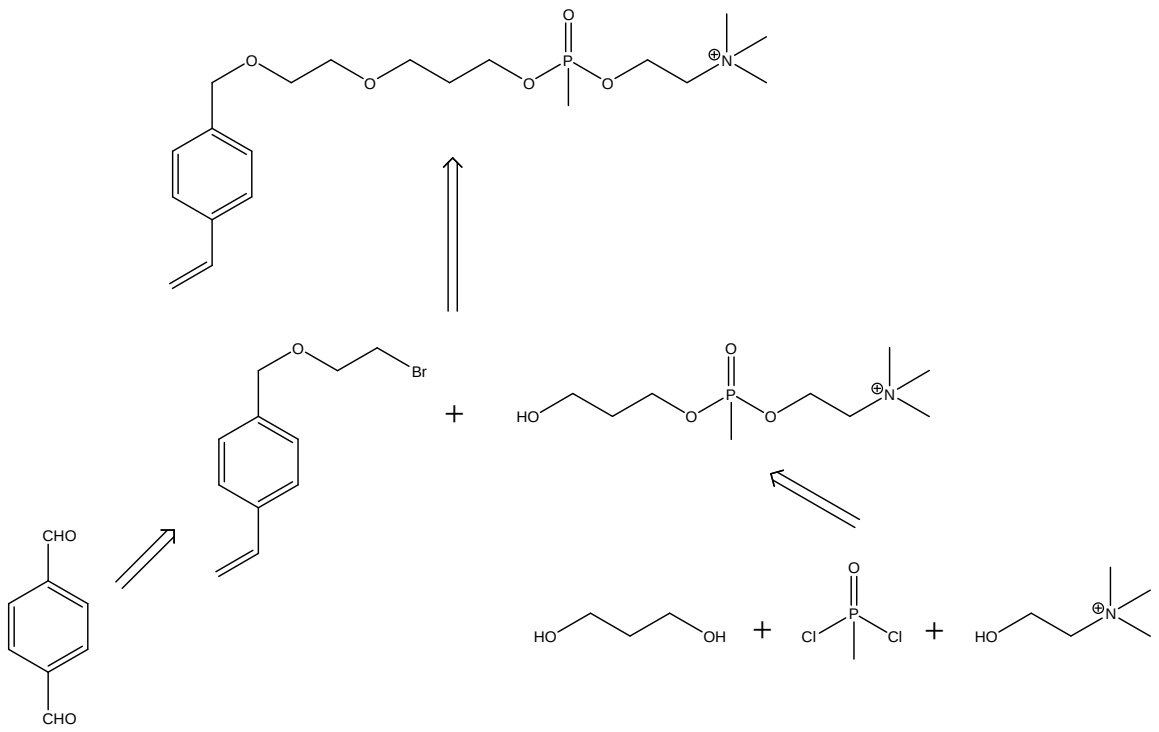
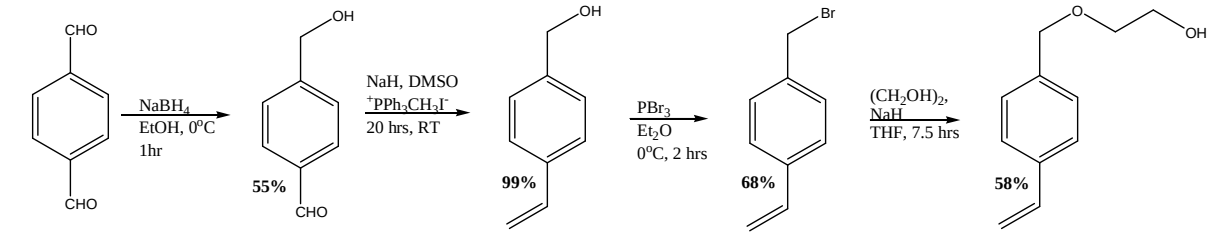


Figure 7: Retrosynthesis

Scheme 1: Synthesis of 2-(4-vinylbenzyloxy)ethanol



Terephthalaldehyde — 4-(hydroxymethyl)benzaldehyde — (4-vinylphenyl)methanol — 1-(bromomethyl)-4-vinylbenzene — 2-(4-vinylbenzyloxy)ethanol

JOC **2001**, *66*, 4476 — *Tetrahedron*, **2005**, *61*, 12160 — *Macromolecules*, **2002**, *35*, 9466

70.0% Average Yield

21.5% Process Yield

Previous syntheses of (4-vinylphenyl) methanol have been conducted using styrene as a starting material. However, multiple steps are involved and the yield suffers accordingly.[13,14] Thus, starting from inexpensive and readily available terephthalaldehyde appeared to be

advantageous. Initial attempts at a mono-reduction to 4-(hydroxymethyl)benzaldehyde employed lithium aluminum hydride. Despite varying equivalents of the reagent (1.00, 0.50, and 0.25 eq), the reactions all resulted in over-reduction. Employing sodium borohydride in tetrahydrofuran[15] afforded a mixture of the monohydroxy and dihydroxy products. Employing ethanol as a solvent afforded a mixture of starting material and the monohydroxy product in a satisfactory yield.

(4-vinylphenyl)methanol was synthesized by performing a Wittig reaction upon 4-(hydroxymethyl)benzaldehyde.[16,17] The ylide was prepared by reacting triphenylphosphine with methyl iodine in benzene. Reaction optimizations lead to the use of two equivalents of the activated ylide. Presumably, the hydroxyl group is deprotonated by the first equivalent, with the second reacting normally with the substrate. Upon purification, the desired compound was obtained in very high yield. It is interesting to note that attempts to conduct a Wittig reaction with terephthalaldehyde failed to react.

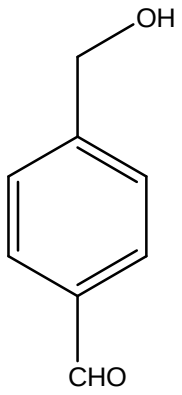
Use of freshly distilled tribromophosphine resulted in the formation of the desired bromine[17] in a fair yield, which was then reacted with ethylene glycol to afford 2-(4-vinylbenzyloxy)ethanol.[18]

Future Endeavors

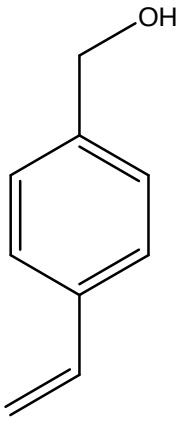
Focus is now shifting to the formation of the phosphonate ester fragment. Methylphosphonic dichloride has been successfully reacted to form phosphonate esters with a variety of alcohols.[19-21] Additionally, recent literature indicates that the process may be facilitated by catalysts such as triazole. Upon completion, this fragment will be attached to the styrene-linker previously developed.[22,23] Polymerization will be induced utilizing techniques established for styrene polymerization.[24]

Experimental

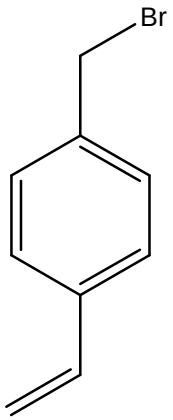
General Comments: Chemicals employed were obtained from commercial sources. Reactions were typically monitored at bench via thin-layer chromatography. Routine structure analysis was conducted utilizing 400MHz ^{1}H-NMR to confirm agreement with reported literature data.



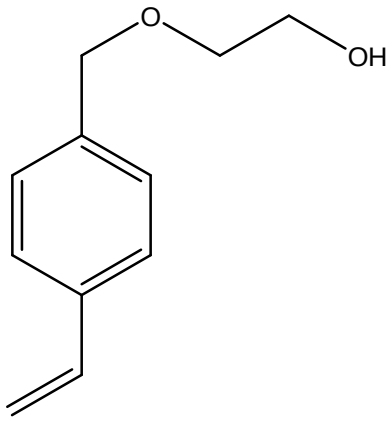
4-(hydroxymethyl)benzaldehyde: 2.000g of terephthalaldehyde (14.92 mmol) was placed in a flame-dried, nitrogen-purged round bottom flask with a stir bar. The compound was dissolved in 44.8 mL of 100% ethanol and chilled to 0^{o}C. After cooling, 0.202g of sodium borohydride (5.22 mmol) was added to the flask. After one hour the reaction was quenched with water, worked up in ethyl acetate, dried with magnesium sulfate, and the solvent removed. Purification via silica column chromatography utilizing a 50/50 mixture of ethyl acetate and hexanes afforded 1.110 grams (55% yield) of the desired product as white crystals. ^{1}H-NMR was in accordance with previously reported data.[15]



(4-vinylphenyl)methanol: 2.20 mL of distilled DMSO was placed in a flame-dried nitrogen-purged 5mL flask, into which 0.035g of dried sodium hydride was added. 0.594g (1.470 mmol) of methyl ylide was then added to the mixture and allowed to stir for 30 minutes. 100mg (0.735 mmol) of 4-(hydroxymethyl)benzaldehyde was then added to the mixture. After 22 hours, the reaction appeared to have run to completion as indicated by bench-top TLC. The reaction was quenched using a saturated solution of ammonium chloride, worked up in ethyl acetate, dried using magnesium sulfate, and the solvent removed via rotary evaporation. Silica gel column chromatography utilizing a 50/50 mixture of ethyl acetate and hexanes resulted in the recovery of 98mg of product (99% yield) in the form of an light-colored oil. ^{1}H-NMR was in accordance with previously reported data.[17]



1-(bromomethyl)-4-vinylbenzene: 50mg (0.373mmol) of (4-vinylphenyl)methanol was placed in a flame-dried nitrogen-purged 10mL flask. 4.14mL of diethyl ether was added and the mixture chilled to 0^{o}C. After cooling, 0.055mL of tribromophosphine (0.5595 mmol) was added and allowed to react for one hour, after which another 055mL of tribromophosphine (0.5595 mmol) was added and stirred for another hour. The reaction was then quenched with water and a saturated solution of sodium bicarbonate, worked up in diethyl ether, rinsed with brine, dried with magnesium sulfate and the solvent eliminated via rotary evaporation. Silica gel column chromatography was employed to purify the compound using an 80/20 mixture of hexanes and ethyl acetate. 50mg of the desired product was recovered (68% yield) as a reddish oil. ^{1}H-NMR was in accordance with previously reported data.[17]



2-(4-vinylbenzyloxy)ethanol: 1.18mL of ethylene glycol was added to a 5mL flame-dried, nitrogen purged round bottom flask with a magnetic stir bar. A mixture of THF and 0.010g of sodium hydride was then injected. A mixture of 0.021g of 1-(bromomethyl)-4-vinylbenzene (0.1065mmol) and the remaining THF (total of 0.11mL) was injected into the flask. The temperature was increased to 60^{o}C via an oil bath. After 7.5 hours the reaction was cooled, quenched with water, worked up in chloroform, dried with magnesium sulfate, and the solvent removed via rotary evaporation. Silica gel column chromatography was performed using an 80/20 mixture of hexanes and ethyl

acetate and afforded 11mg of the product (58% yield) as a clear, colorless oil. ^{1}H-NMR was in accordance with previously reported data.[18]

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